

DEVELOPMENT OF A FAST BETA-AGONIST ELISA FOR THE SCREENING OF MILK WITH MINIMAL SAMPLE PREPARATION

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INTRODUCTION

Beta-agonists can be used for a number of purposes including therapeutic treatment and for promoting growth. In the European Union, the use of these compounds for growth promotion in farm animals intended for human consumption is banned, as there is a potential health risk to consumers. These compounds are listed for monitoring in live animals and animal products.

The availability of rapid screening methods to detect beta-agonists facilitates the testing process. This study reports the development of a fast beta-agonist Enzyme-Linked Immunosorbent Assay (ELISA) for the rapid screening of milk with minimal sample preparation.

METHODOLOGY

Competitive ELISA

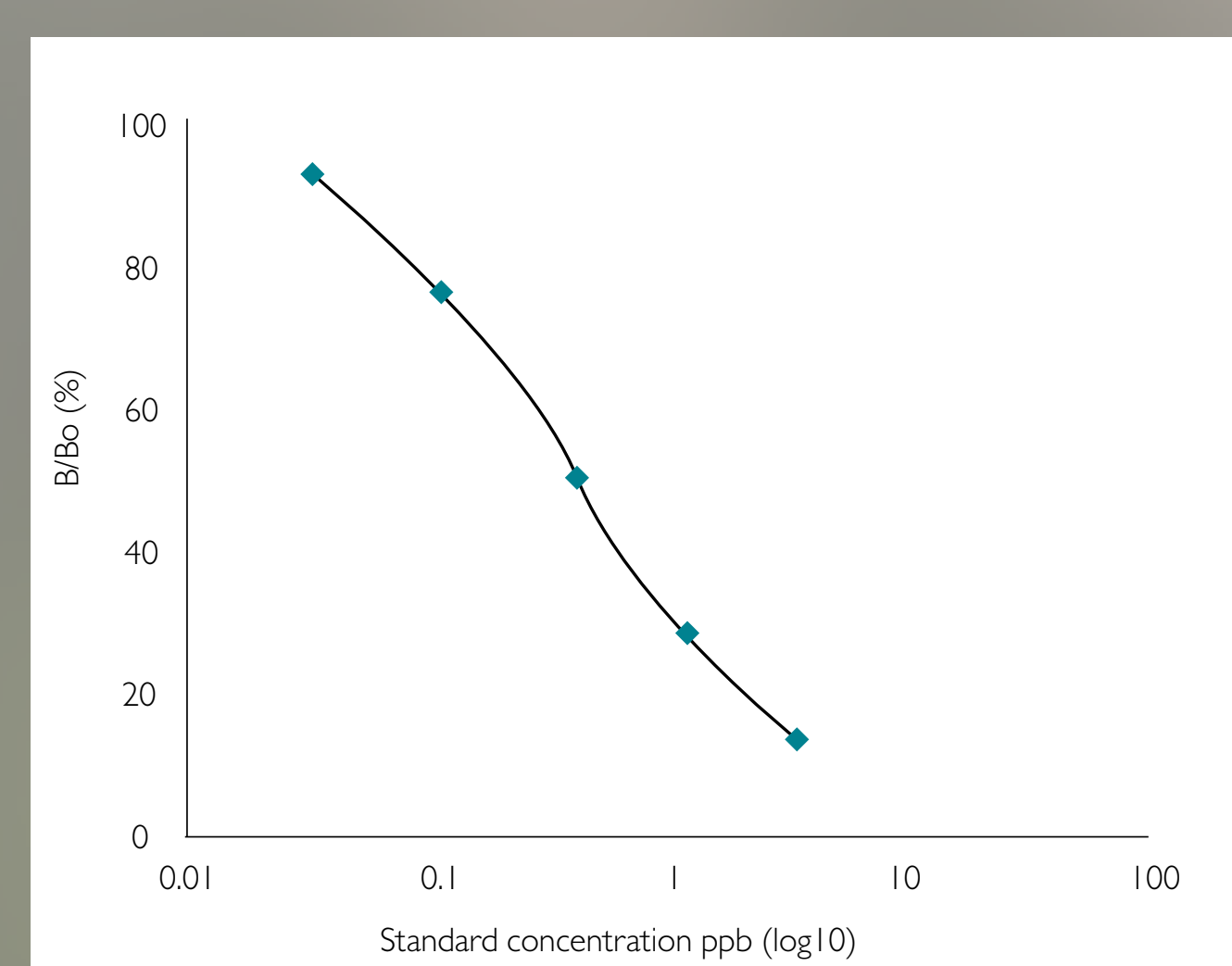
Quantitative determination of the target analyte was carried out using Beta-agonist Fast Milk ELISA kit (BF3507, Radox Food Diagnostics, Crumlin, UK). The kit components in this competitive ELISA are ready to use, with a 96-well breakapart pre-coated microtitre plate, six liquid standards and a working strength conjugate. The assay was performed in accordance with the manufacturer's instructions.

Milk Sample Preparation

Minimal sample preparation is required: chilled milk samples are prepared for analysis by carrying out a single centrifugation step before the sample is applied directly to the microtitre plate. Typically forty samples can be analysed in less than 1 hour when run in duplicate. The sample volume required for each well is 100µl.

RESULTS

Typical calibration curve for clenbuterol



Sensitivity

Analyte	Matrix	LOD (ppb)
Clenbuterol	Milk	≤0.1

14.065.109

Specificity/Cross-reactivity (CR)

Analyte	CR (%)
Clenbuterol	100
Cimbuterol	125
Salbutamol	115
Tulobuterol	97
Bromochlorbuterol	86
Brombuterol	75
Carbuterol	56
Mabuterol	50
Clenpenterol	33
Terbutaline	33
Mapenterol	30
Pirbuterol	28
Penbutolol	6
Cimaterol	5
Clenproperol	5
Clorprenaline	2
Hydroxymethyl clenbuterol	1
Fenoterol	<1
Zilpaterol	<1
Deisozilpaterol	<1
Ractopamine	<0.0125

13.1053.1076.421; 14.1494.1495.421

Recovery

Sample	Mean Recovery (%)
1	102
2	110
3	121

14.069.109

Intra-assay precision

Plate	Level 1 CV (%)	Level 2 CV (%)	Level 3 CV (%)	Level 4 CV (%)	Level 5 CV (%)	Level 6 CV (%)
1	4.3	6.3	4.1	4.1	3.4	5.4
2	5.3	5.2	3.6	4.6	4.7	8.8
3	6.5	5.8	6.9	4.7	3.4	4.8
4	6.3	4.8	3.8	3.4	3.2	6.1

14.1582.421

CONCLUSION

The results indicate optimal analytical performance of this user-friendly Beta-agonist Fast Milk ELISA, for the rapid screening of beta-agonists in milk samples. The ready-to-use reagents, along with the simple one-step sample preparation method and short incubation times, minimises the time required to generate results: less than 1 hour for 40 samples run in duplicate. This does not compromise the assay precision as the CV (%) values were typically < 9% for different concentration levels.

The mean recovery was in the range, 102 –121%. The combination of ready-to-use components, short incubation times and low limit of detection (≤0.1 ppb) make this ELISA a cost-effective, robust, precise, analytical tool for the screening of beta-agonist drugs in milk when a large number of test samples have to be analysed to monitor legislative compliance.